

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of the Claims

Claims 1-22 (Cancelled)

Claim 23 (Currently Amended): A process for the ~~preparation of~~ preparing an agglutination reagent for rapid and early detection of detecting typhoid comprising:

- (a) preparing ~~an a~~ a polyclonal-monospecific antibody specific to *Salmonella typhi*;
- (b) preparing a latex particle suspension; and
- (c) coating ~~of a~~ latex particle with ~~the said~~ a polyclonal-monospecific antibody specific to *Salmonella typhi*;

wherein said polyclonal-monospecific antibody specific to *Salmonella typhi* is prepared according to a method comprising:

- (i) ~~cloning a Flagellin gene sequence specific to *Salmonella typhi*, expressing said Flagellin gene sequence by recombinant DNA technology thereby expressing a recombinant protein, followed by purifying said recombinant protein by affinity chromatography thereby forming a purified recombinant protein, raising a hyper immune sera against said a purified recombinant protein encoded by a Flagellin gene specific to *Salmonella typhi* in an animal, and~~
- (ii) ~~separating an said polyclonal-monospecific antibody (immunoglobulin) fraction from said hyper immune sera by precipitating in an ammonium sulphate, suspending in a 50 mM phosphate buffer of pH 7.2, and dialyzing;~~

wherein said latex particle suspension is prepared according to a method comprising:

- (i) ~~mixing 1% carboxylated latex particles of size 0.88 to 0.90 μ m and a 40 mM 2-N morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1 on a vortex mixer for about 60 seconds, centrifuging at 10,000 rpm for 10-12 minutes at about 4°C, followed by washing twice with a 20 mM MES buffer of~~

pH 5.5 at 10,000 rpm for 10-12 minutes at about 4°C, sonicating by a tip sonicator at about 5 watts for 60-120 seconds, thereby forming a sonicated washed latex particle;² and

- (ii) adding drop wise a freshly prepared solution of a 0.1 M 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in a 20 mM MES buffer of pH 5.5 to said sonicated washed latex particles obtained from step (i) above in a ratio of 1:1 while vortexing slowly thereby forming a suspension, rotating the suspension slowly end-over-end for about 3 hours at a temperature of 20-25°C, washing thrice with a 20 mM MES buffer (pH 5.5); and followed by

- (iii) sonicating said suspension by a tip sonicator for 60-120 seconds at about 5 watts; and

wherein said latex particle is coated by according to a method comprising:

- (i) adding-reacting 0.6-1.0 mg per ml of said polyclonal-monospecific antibody (immunoglobulins) fraction to with said suspension washed latex particle thereby forming an antibody particle mixture, rotating said antibody particle mixture end-over-end for 18-20 hours at a temperature of about 20-25°C thereby forming a solution comprising an a polyclonal-monospecific antibody coated latex particle,
- (ii) stopping the reacting step (i) coating reaction by adding 1M glycine (pH 11.0) taken in quantity of 0.06 ml per ml of said solution, followed by centrifugation at 10,000 rpm for 10-12 minutes at a temperature of about 4°C, and
- (iii) washing thrice said antibody coated latex particle polyclonal-monospecific with a washing buffer comprised of 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide, suspending in a storage buffer to a final concentration of 1%, sonicating for about 60 seconds at about 5 watts and storing at 4°C.

Claim 24 (Currently Amended): An agglutination reagent for rapid and early detection of typhoid, comprising of 1% a carboxylated latex particles coated with an antibody specific to *Salmonella* typhi, suspended in storage buffer.

Claim 25 (Previously Presented): The agglutination reagent as claimed in claim 24, wherein the size of the said latex particles is 0.88 to 0.90 μm .

Claim 26 (Previously Presented): The agglutination reagent as claimed in claim 24, wherein the said storage buffer is comprised of 50 mM glycine pH 8.5, 1.0% bovine serum albumin, 0.03% surfactant, 0.1% sodium azide and 0.01% thimerosal.

Claim 27 (Currently Amended): The agglutination reagent for rapid and early detection of typhoid as claimed in claim 24, wherein ~~the said antibody is the~~ an immunoglobulin fraction of the a hyper immune sera raised in said animals against the a recombinant protein encoded expressed by cloning of a Flagellin gene sequence specific to *Salmonella* typhi by recombinant DNA technology, and wherein said storage buffer is a suspended in 50 mM phosphate buffer.

Claim 28 (Withdrawn): A kit for rapid and early detection of typhoid comprising 1% agglutination reagent as claimed in claim 24 suspended in storage buffer, glass slides, droppers, wooden sticks and positive and negative controls.